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Protocol status: Working
 We use this protocol in our group and it is working.

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Vibrio natriegens electrocompetent preparation

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ABSTRACT

This is an approach to electroporate DNA into *Vibrio natriegens* wt and *Vibrio natriegens* sp. Vmax. It's generally pretty similar to canonical electroporation protocols with exception to the wash buffer and temperature of activity.

Essentially pulled straight from Weinstock *et al.*

MATERIALS

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Potassium chloride **P212121**

Magnesium Chloride **Fisher Scientific Catalog #AC223210010**

Sucrose **Contributed by users**

Potassium phosphate, dibasic, anhydrous **Bio Basic Inc. Catalog #PB0447.SIZE.500g**

Brain Heart Infusion broth (BHI) **Merck Millipore (EMD Millipore) Catalog #1.10493.0500**

STEP MATERIALS

Brain Heart Infusion broth (BHI) **Merck Millipore (EMD Millipore) Catalog #1.10493.0500**

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Reagents list list for wash buffer, BHI, and BHI with v2 salts.

Keywords: *Vibrio natriegens*,
Vmax, electroporation

Media recipes

1 BHI media stock

Brain Heart Infusion broth (BHI) Merck Millipore (EMD Millipore) Catalog
#1.10493.0500

2 BHI + v2 salts

Brain Heart Infusion broth (BHI) Merck Millipore (EMD Millipore) Catalog
#1.10493.0500

- [M] 204 millimolar (mM) NaCl
- [M] 4.2 millimolar (mM) KCl
- [M] 23.14 millimolar (mM) MgCl₂
- [M] 680 millimolar (mM) Sucrose

Filter sterilize or autoclave

3 Electroporation buffer

- [M] 680 millimolar (mM) sucrose
- [M] 7 millimolar (mM) Potassium phosphate dibasic
- pH7

Filter or autoclave to sterilize.

Electrocompetent prep

4 **** The day before****

Inoculate 10 mL [BHI + v2 salts] with *V. natriegens* overnight culture at 30 °C with agitation at 200 r.p.m.

5 **** The day of ****

On the following day, prepare 60mL of [BHI + v2 salts] in a baffled flask.

Generally, 60mLs of outgrowth yields one electrocompetent cell aliquot.

- 6 Inoculate media with overnight at a dilution of 1:100 to 1:200 (overnight culture/fresh medium). Incubate culture with shaking at 37 °C shaking at 200r.p.m. until an OD600 of 0.5.
- 7 4 °C (operator can be at RT, just cells should be chilled)
Split outgrowth into two chilled 50-mL falcon tubes and incubated on ice for 15min.
- 8 4 °C
Pellett cells at 4 °C, spinning at 5000 rcf for 10mins.
- 9 4 °C (operator can be at RT, just cells should be chilled)
Carefully decant supernatant and gently resuspend cell pellett in at lesat 1mL of electroporation buffer (680 mM sucrose, 7 mM K2HPO4, pH 7).
- 10 4 °C (operator can be at RT, just cells should be chilled)
Top off centrifuge tube (in most cases a 50mL falcon tube) with electroporation buffer. Gently mix
- 11 4 °C (on ice)
Pellett cells at 4 °C, spinning at 5000 rcf for 10mins.
- 12 4 °C (operator can be at RT, just cells should be chilled)
Carefully aspirate away supernatant with a pipette (pellett will be looser than before).
Get volume down to around 1-4mL, pellett does not have to be super dry.
- 13 4 °C (operator can be at RT, just cells should be chilled)
Repeat steps 8,9,10 at least three times for a minimum of three washes.

After pelleting after final wash, carefully pipette off as much of the supernatant as possible.

- 14** 4 °C (operator can be at RT, just cells should be chilled)
Resuspend pellet so final volume of cells + electroporation buffer is about 50-100uL.

- 15** To check concentration of, generally a 1:1000 inoculation into BHI media should have an OD of 0.15 +/- 0.02. If too dilute, spin down as before and resuspend in a smaller volume.

Storage

- 16** For long term storage. Snap freeze cell aliquots in dry ice bath or liquid nitrogen. 50uL per electrocompetent aliquot. Store at -80.

Electroporation

- 17** Thaw
Transfer aqueous electrocompetent aliquot to a chilled 0.1cm gapped cuvette on ice.
- 18** Apply 0.9 kV. So far tested in a biorad micropulsar.

Resuspend cells in 500uL of [BHI+v2 salts]
- 19** Outgrow transformation resuspension at 37 °C (or 30C depending on plasmid) with shaking or rotation.

Plate outgrowth onto LB plates with selection.